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Sex-related responses in rhizosphere processes of dioecious *Populus cathayana* exposed to drought and low phosphorus stress

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Running title: Sex-specific rhizosphere processes in poplar

Highlights

- Effects of drought and P deficiency on diecious *P. cathayana* were investigated.
- Females were more sensitive to drought and P deficiency stresses than males.
- P application ameliorated drought stress more in males than in females.
- P application greatly enhanced root exudation of males under drought stress.
- Greater changes in soil microbes of males may be responsible for higher resistance.

Abstract Extensive research has shown that dioecious plants exhibit sexual dimorphism under extreme environments. However, sex-specific differences in responses to drought, phosphorus (P) shortage or their combination are less known. In our study, impacts of drought, P shortage and their combination on the performance of *Populus cathayana* males and females were investigated. Drought and P deficiency caused a greater negative impact on female growth than on male growth. P application ameliorated the more negative effect of drought on the shoot dry matter accumulation and P concentration in male leaves, while smaller effects were observed in females. The concentration of citrate in the rhizosphere of males was higher under drought combined with P application than under adequate water availability, and the increase was greater in males than in females. Males also showed a higher abundance of main soil microbial groups, including bacteria, actinomycetes, arbuscular mycorrhizal fungi (AMF), and Gram+ and Gram- bacteria in the rhizosphere, resulting in a more resistant microhabitat. In contrast, the abundance of bacteria and AMF was less in the rhizosphere of females exposed to stress conditions, while saprophytic fungi increased significantly. P enhanced drought resistance more in stress-resistant males but less in females under relatively severe drought stress. Increased drought resistance by P in males might be associated with greater plasticity in rhizosphere processes when compared with females.

Keywords: P shortage; drought; rhizosphere processes; main soil microbial groups; carbohydrates; sexual dimorphism.

1. Introduction

Phosphorus (P) limitation impairs tree growth and development (Netzer et al., 2017; Chen et al., 2018). In most cases, P acquisition is restricted by its low mobility and

availability (Schachtman et al., 1998). Water is an important physiological factor that influences the bioavailability of P: when soil water declines, P acquisition by plants decreases. Water does not only promote the mobility of soil P to the root surface, but it can also improve nutrient absorption and transport through enhanced transpiration flow (Pang et al., 2018b). Alternatively, in dry soils, the application of P fertilizer can enhance plant drought resistance through different mechanisms, including enhancing nutrient absorption, improving root hydraulic conductivity and promoting microbial flora (Singh et al., 2010; He et al., 2017; Yu et al., 2019). Thus, there is an inherent association between soil water and P availability, and the performance of plants.

A series of biochemical, physiological and structural responses is triggered in shoot and root tissues under drought and P deficiency (Suriyagoda et al., 2014; Fort et al., 2015; He et al., 2017). Yet, relatively little attention has been paid on how rhizosphere processes mediate resistance to water and P shortage (Meier et al., 2019). Stress can induce the movement of C from plants to soil (Suriyagoda et al., 2010; Xia et al., 2015; Karst et al., 2017). One strategy to enhance P acquisition is to increase the exudation of carbohydrates (Kidd et al., 2018; Pang et al., 2018a). These evolved strategies have especially important implications under drought conditions when hydraulic continuity is reduced (Suriyagoda et al., 2014). Moreover, soil microbial activities govern the fate of nutrient cycling in soil (Xia et al., 2016). Thus, it is worth assessing the strategies shaped by soil microbial communities to withstand drought or low P stress, as these have also been reported as important means of survival in extreme environments (Nautiyal et al., 2000; Rubin et al., 2017; Orwin et al., 2018). For example, plant-associated functional microorganisms, such as mycorrhizal fungi, P-solubilizing bacteria and plant growth-promoting rhizobacteria (PGPR), enhance plant performance

and promote stress resistance (Wu and Xia, 2006; Jung et al., 2012; Barnard et al., 2013; Ngumbi and Kloepper, 2016; Rubin et al., 2017). Arzanesh et al. (2011) found that *Azospirillum* spp. with P-solubilization activity could survive drought stress. In turn, plant adaptation is positively affected by the presence of *Azospirillum*, which helps plants to tolerate both biotic and abiotic pressures. Thus, the plant rhizosphere can contain microbial communities that produce a wide range of phytohormones or plant growth regulators that may enhance plants' tolerance to, e.g., low P and drought stress.

Dioecious plants meet different reproductive demands and adapt to particular selective pressures (Barrett and Hough, 2012; Lei et al., 2017). Generally, the high reproductive cost of females would reduce their resistance to abiotic stresses, resulting in male-biased sex ratios (Juvany and Munne-Bosch, 2015; Munne-Bosch, 2015; Hultine et al., 2016). For instance, morphological and physiological responses of dioecious *Populus* species have showed sex-specific differences in responses to various environmental factors (reviewed by Melnikova et al., 2017). Also, plant responses to multiple simultaneous stresses differ from the responses to individual stresses (Suzuki et al., 2014). Although water and P can be major limiting factors that affect the productivity of plants, there is little information about the responses of dioecious plants to the interaction of these two factors, when there is a shortage of both of them (Vitousek et al., 2009).

*Populus cathayan*a is a rapidly growing dioecious species, which plays an important role in the afforestation and ecological conservation in northern China. Males display better tolerance to environmental stresses (e.g. drought, salinity, heavy metals, and nutrient deficiencies) than females (Xu et al., 2008; Chen et al., 2010; Zhang et al.,

2014; Li et al., 2016; Liu et al., 2020a, 2020b). Based on this knowledge, we designed growth experiments with low P and drought to investigate the responses of *P. cathayana* females and males according to shoot dry matter accumulation, P concentration in leaves and rhizosphere processes, including citrate concentrations, and the composition of main soil microbial groups and communities. We hypothesized that (1) *P. cathayana* has sexually different responses to drought, low P and their combination, and that males possess a higher stress tolerance than do females, (2) the citrate concentration and the composition of main soil microbial groups and communities are sex-specific in the rhizosphere and dependent on the type of stress, and (3) the mediation of rhizosphere processes are related to the stress resistance of *P. cathayana*.

2. Materials and Methods

2.1. Plant materials and soils

Populus cathayana shoots were collected from 32 different trees of both sexes, sampled from 8 populations (two adult trees of each sex per population) from the Qinghai Province, China (35°56′N, 101°35′E; for detailed information see Xia et al., 2020a). Cuttings (18-20 cm in length, and 2 cm in diameter with 2-4 dormant buds) were rooted according to Xia et al. (2020b). The sandy soil was obtained from the experimental site at the Hangzhou Normal University in Zhejiang, China. Soil properties (per kg): 2.82 g soil organic matter content, 0.28 g total N, 2.62 mg available P, 90.65 mg available K and pH 8.64 (the ratio of soil to $CaCl_2$ solution was 1:2.5). The experiment was conducted in the glasshouse at the Hangzhou Normal University in Zhejiang. The

growth conditions were as follows: a daytime temperature of 21-25 °C, a night-time temperature of 15-18 °C, with 12-14 h light: dark cycle.

2.2. Experimental set-up

In order to investigate how dioecious *P. cathayana* responds to low P and drought, pot experiments were performed in a glasshouse with two sexes and four different growth conditions: well-watered and high P availability (WW/HP) and well-watered and low P availability (WD/LP) to evaluate the effects of P shortage alone; water deficiency and high P availability (WD/HP) to evaluate the effects of water shortage alone, and water deficiency and low P availability (WD/LP) to evaluate the dual effect of water and P shortages. Each treatment included four replicates. We filled the pots with 10 kg of air-dried sandy soil. Before the treatments were established, soil was supplied uniformly with added basal nutrients as follows (mg per pot): $Ca(NO_3)_2 \cdot 4H_2O$ 8040; K_2SO_4 100; $MgSO_4 \cdot 7H_2O$ 130; $MnSO_4 \cdot H_2O$ 50; $ZnSO_4 \cdot 7H_2O$ 75; $CuSO_4 \cdot 5H_2O$ 15. All pots were randomized weekly. Pots in low P-availability treatments (WW/LP and WD/LP) did not receive P application, while pots in high P-availability treatments (WW/HP and WD/HP) had P added to provide non-limiting P availability (2 g P pot^{-1}). In the well-watered treatment, the water content of all pots was weighed every other day and they were re-watered to 75% of the maximum field capacity by replacing the amount of water transpired. In the water deficiency treatment, the pots were watered to 15% of the maximum field capacity. The dry mass was measured after six months (from March to August 2018) and separated into leaves, stems and roots. Following root excavation, the soil adhering to roots was defined as rhizosphere soil. One part of the fresh soil sample was used to measure soil citrate contents, and the other part was used to measure

soil moisture and phospholipid fatty acid (PLFA) parameters under each treatment after freeze-drying. All measurements were calculated in terms of soil dry weight.

2.3. Determination of foliar traits

Five days before the final harvest, the net photosynthetic rate was measured in each treatment for the fourth fully expanded leaf with the Ll-6400 photosynthesis measuring system (Li-Cor, Inc., Lincoln, NE, USA) between 08.00 and 11.30 h. The measuring conditions were as follows: the air flow rate chamber was 500 μmol s^{-1} through the sample, and the leaf temperature was maintained at 25 ± 0.8 °C. The CO_2 concentration of the chamber was mediated by 400 ± 5 μmol mol^{-1}. Then, the selected leaves were weighed and placed in an oven at 105 °C. The leaf water content was calculated as the difference between fresh and dry weights. Also, the specific leaf area (SLA, cm^2 g^{-1}) was estimated as the ratio of the leaf area divided by its dry mass (Xia et al., 2020b). Subsequently, the P concentration of leaves was determined according to the molybdovanadophosphate method (Varian Vista-Pro CCD; Johnson and Ulrich, 1959).

2.4. Determination of citrate and phospholipid fatty acids (PLFA)

Citrate contents in the rhizosphere were determined according to Zhang et al. (2016). Briefly, 5 g fresh rhizosphere soil were homogenized and placed into 50 ml of 0.2 M $CaCl_2$, followed by shaking for 5-10 s to create a homogeneous suspension. A suspension volume of 10 ml was collected for the carboxylation analysis by HPLC. The chromatographic separation was performed on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5 Micrometers; Alltech Associates Inc., Deerfield, IL, USA). The mobile

phase was 25 mM KH_2PO_4 (pH 2.25) with a flow rate of 1 ml min^{-1} at 31 °C. The absorbance was determined at 214 nm.

Soil samples were frozen and used for the determination of the composition of soil microbial communities, assessed by the phospholipid fatty acid (PLFA) method, as described by Xia et al. (2019). The following biomarkers were used: terminal-branched saturated PLFAs (i14:0, a14:0, i15:0, a15:0, i16:0, a16:0, i17:0 and a17:0) represented Gram-positive bacteria, cyclopropyl-saturated PLFAs (16:1 ω9c, 16:1 ω7c, i17:1 ω9c, 17:1 ω8c, 18:1 ω7c, 18:1 ω5c, cy17:0 ω7c and cy19:0 ω7c) indicated Gram-negative bacteria, saprophytic fungi (18:1 ω9c and 18:2 ω6c), arbuscular mycorrhizal fungi (AMF) (16:1 ω5c) and actinomycetes (16:0 10-methyl, 17:0 10-methyl and 18:0 10-methyl). The sum of the Gram-positive bacteria (Gram +), Gram-negative bacteria (Gram -) and non-specific bacteria (14:0, 15:0, 15:0 DMA, 16:0, 17:0, 18:0 and 20:0) was selected as indicators of the total bacteria (Frostegård and Bååth, 1996).

2.5. Statistical analysis

The foliar traits, shoot dry matter accumulation, citrate concentrations and main soil microbial groups were analyzed with three-way analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Chicago, IL, USA). All treatments were regarded as fixed factors (i.e. sex, water or P treatments as well as their interactions). Subsequently, differences in the foliar traits, shoot dry matter accumulation, citrate concentrations and main soil microbial groups between females and males were identified by independent-samples t-test. To estimate stress effects on females and males, we calculated an abiotic stress intensity index (range = 0-1) based on the shoot dry matter production for each

growth condition (water-deficiency, P-shortage, water-deficiency and P shortage): Abiotic stress intensity index = ($P_{T-S} - P_{T+S}$) /P_{T-S}, where for a specific gender, P_{T-S} is the mean shoot dry matter accumulation of plants grown without a shortage of the resources considered and P_{T+S} is the mean shoot dry matter accumulation of plants grown with a shortage of the resources considered. The higher the index, the stronger was the effect of the shortage (Fort et al., 2015). T-test was selected to compare sexual differences of stress intensity indices for each growth condition at a level of significance of $P < 0.05$. The composition of main microbial groups was also evaluated by the principal coordination analysis (PCoA) (Canoco 5.0). The statistical significance of differences between data sets was assessed by PerMANOVA using the weighted PCoA scores in PAST (http://folk.uio.no/ohammer/past/). Pearson correlation analysess with bivariate correlation coefficients were performed to show relationships among foliar traits, shoot dry matter accumulation, citrate concentration and main soil microbial groups.

3. Results

3.1. Foliar traits and growth performance in females and males

Water deficiency or P shortage reduced foliar traits including leaf water, specific leaf area, net photosynthetic rate and foliar P concentration in both males and females, whereas sex significantly affected only foliar P concentration (Table S1). For instance, no significant difference was found in leaf water and specific leaf area between females and males, irrespective of growth conditions (Table S1 and Figure 1a-b). However, the

differences in net photosynthetic rate and foliar P concentration between females and males depended on the growing environment (Figure 1). When water and P were sufficient, the foliar P concentration of females was significantly higher than that of males (Figure 1d). Conversely, males significantly surpassed females in net photosynthetic rate and foliar P concentration under water deficiency and high P supply (Figure 1c-d). In addition, no sex-specific differences were observed between well-watered and P shortage or water deficiency and P shortage conditions.

Stress effects showed sex-specific differences. Females were much more vulnerable to stress when compared with males (Figure 2). Dual limitation had a greater negative impact on growth than drought or low P alone. Particularly, the shoot dry matter accumulation of females was significantly higher than that of males, except for drought and high P condition (Figure 2). Under soil water shortage, P application significantly increased the shoot dry matter accumulation of males, but it had less effect on that of females.

3.2. Variation in rhizosphere processes between males and females: citrate concentrations and main soil microbial groups

Citrate was detected in all treated rhizosphere soils of both males and females. However, the concentrations varied significantly depending on sex, water availability, P availability, water availability × P availability, and sex × water availability × P availability (Table S2). The citrate concentration of the female rhizosphere soil was lowest when water and P were sufficient, but it increased under drought conditions. In males, the citrate concentration of the rhizosphere soil was higher than that of females,

especially under stress conditions. Our analysis revealed that the shortage of P resulted in a sharp increase in the citrate content in the male rhizosphere soil under sufficient moisture. On the other hand, the release of citrate was promoted most strongly by the application of P under drought (Figure 3).

Compared to the effects of a single factor (sex, water or P), the interactions between sex and water availability or water availability and P availability affected most main soil microbial groups (Table S2). Under well-watered conditions, the composition of soil microbial groups including bacteria, saprophytic fungi, actinomycetes, Gram+ and Gram- were not significantly different between females and males (Figure 4a-f). The presence of the only AMF biomarker in males was significantly higher than that in females under well-watered conditions combined with P shortage (Figure 4d). On the other hand, under water deficiency but high P supply, male rhizosphere was characterized by higher contents of bacteria, AMF, actinomycetes, Gram+ and Gram- when compared to females (Figure 4a, c-f). Surprisingly, females contained far more saprophytic fungi than males in this growth condition (Figure 4b). Males had higher Gram+ and Gram- levels than females under dual water and P limitations (Figure 4e-f).

The PCoA results of soil microbes in the male and female rhizospheres exposed to P or water shortage and their interaction are shown in Figure 5 a-b. The first and second principal component explained 83.9% of variation in females and 85.1% of variation in males. The PerMANOVA test based on the Bray-Curtis distance measures showed that the soil microbial community structure was significantly different in both females ($F=3.3$, $P<0.05$) and males ($F=5.2$, $P<0.01$) among these clusters grouped by water and P interactions (Table S3). Specially, under high P conditions, the soil water content

significantly influenced microbial community structures of both males and females. By contrast, under water deficit conditions, the P supply level significantly affected the microbial community structure of females. Also, under changes in both soil P and moisture, the soil microbial community of males became significantly altered (Table S3).

3.3. Relationships among foliar traits, growth performance, citrate concentration and main soil microbial groups under different water and/or P conditions

A significant positive correlation was found between shoot dry matter production and foliar traits, whereas citrate concentrations in the rhizosphere were negatively correlated with shoot dry matter accumulation and foliar P concentrations (Table 1). On the other hand, we found no relationships among main soil microbial groups and foliar traits or shoot dry matter accumulation, with an exception between saprophytic fungi and net photosynthetic rate. Moreover, there were significant positive relationships between citrate concentrations in the rhizosphere and bacterial PLFAs, Gram+ or Gram- PLFAs (Figure 6a, c-d), whereas no significant relationships were observed between rhizosphere citrate concentrations and saprophytic fungi PLFAs (Figure 6b).

4. Discussion

4.1. Sex-specific responses of P. cathayana to P and water interactions

Our study indicated that drought and low P significantly reduced foliar traits and shoot dry matter accumulation. Although some studies have shown that fertilization can partly mitigate the damage effects of drought, P application did not completely alleviate the negative effects of drought in our studies (Afshar et al., 2014; He et al., 2019). Still, drought exerts great damage on numerous biochemical and cellular processes, which cannot be overcome just by P application (Burman et al., 2009). On the other hand, plant roots only adsorb P in the inorganic form, and limited soil water restricts external P to reach the root surface, thereby affecting P assimilation. This can be confirmed by the presence of a relatively low P concentration in leaves under drought.

There was sex-specific variation in the ability to resist stress. Although there was a significantly positive correlation between foliar P concentration and shoot dry matter accumulation, we still observed their independent responses to P and water interaction in females and males. Generally, females need more P to support and maintain higher P utilization efficiency for fast growth to match their higher reproductive costs (Xia et al., 2020a). In most cases, females accumulate more dry matter than males. However, P application under drought changed this trend: males and females did not differ in shoot dry matter accumulation, possibly because males absorbed more P. Compared with females, males did not possess higher biomass under well-watered/high P conditions, but they were less impacted by stress. For instance, P application under drought significantly increased the shoot dry matter accumulation of males, and the performance of females was greatly reduced under drought stress when compared with the more resistant males.

4.2. Sex-specific rhizosphere processes under combined drought and P stress

Stress can induce exudation for C movement from a plant to soil with positive effects on plant growth (Hirano et al., 2012; Desai et al., 2014; Karst et al., 2017; Guo et al., 2019). Under low P stress, plants can enhance P availability by hydrolysis and mineralization of organic-P substrates via the release of Pi-solubilizing low-weight organic acids (LOA) and phosphatases, which can liberate free Pi with a higher efficiency (Lambers et al., 2009; Richardson et al., 2011). Yet, the interactive effect of water and P shortage on the rhizosphere carboxylate concentration and composition has been studied only little. Suriyagoda et al. (2010) found that severe drought stress causes the roots of several legume species to exude more carboxylates into the rhizosphere than when adequately watered. Moreover, citrate is the most effective carboxylate to increase P solubility, regardless of the soil type (Hinsinger et al., 2018). In the current study, we discovered that citrate release is induced under drought or P limitation. LOA exudation increasing under drought, low P or their combination may enhance access to extra P in adverse environments. However, compared with females, we found that males have more effective rhizosphere processes, thereby having a stronger capacity to increase the release of citrate to mobilize Pi for improving P nutrition. As a consequence, we found that under drought, the application of P largely promotes the exudation of carboxylates. A previous study has suggested that drought stress induces carboxylate exudation in pine seedlings, but it also depends on the magnitude of drought (Reid 1974; Reid and Mexal 1977; Karst et al. 2017). Both previous investigations and our observations suggest that P application mitigates drought and promotes citrate release in males. In contrast, females show less ability to produce and release carboxylates to resist abiotic stresses. Thus, plants with great physiological plasticity may have an advantage when they confront nutrient deficiency or other adverse abiotic environments

(Zemunik et al., 2015). It deserves further consideration that the content of rhizosphere carboxylates during drought may be affected by the magnitude of drought, rhizosphere microorganisms and plastic plant responses (Suriyagoda et al., 2014).

Drought and P deficiency influence C flux to soil through changes in rhizodeposition (Reid, 1974; Lyu et al., 2016; Kidd et al., 2018). This root-derived C flux not only promotes substantial nutrient release from soil in extreme conditions, but it may also alter biological processes of soil by modifying microbial communities that affect drought or P shortage resistance (Karst et al., 2017). Kwapata and Hall (1985) found that even under mild to moderate drought contexts, AMF colonization can occur on a substantial level. In our study, males not only promoted the growth of AMF under adequate moisture combined with low P, but also under drought combined with added P. Thus, the symbiosis between males and AMF may compensate plants' P shortage and enhance drought resistance. On the other hand, the load of root exudates is often increased under abiotic stress, which exerts a great impact on the main soil microbial groups (Karst et al., 2017; Vives-Peris et al., 2018). Similarly, we found a significant positive correlation between citrate concentration and rhizosphere bacteria, but not saprophytic fungi. The bacteria were more sensitive in LOA utilization because of high demand for anabolic products (Gunina et al., 2014). Both plants and microorganisms could synthesize and release LOA in the soil environment as an adaptation mechanism in response to nutrient shortage and osmotic differences (Adeleke et al., 2017).

Although we were unable to distinguish, which bacterial populations associate with resistance in males, we did observe an increase in the abundance of main bacterial groups, especially when P was added during drought. Indeed, plant growth-promoting

rhizobacteria (PGPR) may help plants to resist drought stress via the production of a series of phytohormones or plant growth regulators (Ngumbi and Kloepper, 2016; Rubin et al., 2017). The proposed physiological mechanisms include an increased water and nutrient uptake, a synthesis of osmolytes, an increase of antioxidant enzymes and a manipulation of phytohormones (Ngumbi and Kloepper, 2016). Also, an accumulation of Gram-positive bacteria and actinomycetes was found in this situation. Gram-positive bacteria usually show higher stress tolerance compared with gram-negative bacteria (Orwin et al., 2018). Similarly, actinomycetes as a copiotrophic group show a high drought tolerance (Fierer et al., 2012; Barnard et al., 2013). Taken together, the accumulation of these organisms can partly promote the strong assistance of males under drought stress. Also, the saprophytic fungi were largely accumulated in the rhizosphere of females under drought combined with added P. The above-mentioned study showed that females possess acquisitive traits, such as a higher specific root length (SRL) compared with males (Xia et al., 2020a). This consumptive strategy may incur great costs, since such roots often have a fast turnover with short lifespans. On the other hand, pathogenic fungi always infect a host with acquisitive root traits under extreme environments (Freschet et al., 2018). In brief, a large accumulation of pathogenic microorganism in the rhizosphere may be responsible for female growth restriction. On the other hand, the results of the multivariate analysis showed that both males and females were able to adjust the soil microbial community to adapt to different water and P interactions. However, under a specific stress, *P. cathayana* still showed sex-specific adaptation strategies that depended on the adjustments in soil microbial communities.

The differences in reproductive costs between males and females lead to increased

sexual dimorphism under drought or low P stress (Xu et al., 2008; Zhang et al., 2014). In this study, we found, for the first time, that abiotic stress can change the rhizosphere processes in a sex-specific way. This was proved by the observed secretion of carboxylates and changes in the main soil microbial groups. It is noteworthy that a high degree of independence was found between main soil microbial groups and plant performance. Sex-specific adaptation to abiotic stress may be regulated by integrated factors (Zhang et al., 2019). In our study, we did not evaluate changes in root morphology and system architecture, which may be more critical for interpreting and predicting the sexual performance under different water and P interactions. Consequently, such stress responses may partly explain the performance of dioecious plants and differences between sexes in stress resistance. In the future, the response processes and adaptation mechanisms of roots, root exudates and microorganisms to adverse conditions need to be considered comprehensively.

5. Conclusions

We conclude that the performance of *P. cathayana* males and females is greatly affected by drought, low P and their combined effect. Females are more responsive and suffer from greater negative effects than do males when grown under environments with drought stress and low P. We observed that drought resistance increases under P application more in males but less in females. Enhanced stress resistance in males might be associated with the exudation of carboxylates and the changes in the main soil microbial groups.

Author contributions

Zhichao Xia had the main responsibility for data collection, analysis and writing, Yue He and Bin Zhou performed the experiment, Helena Korpelainen contributed to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Pearson correlations between shoot dry matter accumulation, foliar traits and rhizosphere processes in *P. cathayana* females and males under four growth conditions.

	Shoot dry matter accumulation	Leaf water	Specific leaf area	Foliar P concentration	Net photosynthetic rate
Shoot dry matter accumulation	1	0.70**	0.48**	0.91**	0.78**
Citrate concentration in rhizosphere soil	-0.53**	-0.30	0.03	-0.37**	-0.27
Bacteria	-0.23	-0.21	0.06	-0.11	-0.06
Saprophytic fungi	-0.27	-0.22	-0.08	-0.26	-0.37**
Actinomycetes	-0.11	-0.12	0.05	0.05	0.09
AMF	-0.07	0.18	0.21	0.10	0.29
Gram+	-0.22	-0.26	0.10	-0.12	-0.07
Gram-	-0.17	-0.23	0.09	-0.08	-0.03

Correlation coefficients (r) are calculated by pairwise comparisons.* indicates significantly different at $P < 0.05$; **, $P<0.01$.

Figure legends

Figure 1 Foliar traits (means ± SE) of *P. cathayana* females and males under two water treatments [well-watered (WW) and water deficiency (WD)] and two P levels [202.6 (HP) and 2.6 (LP) mg P kg^{-1} dry soil] (a-d). ∗ denotes significant differences between males and females: ∗, $P < 0.05$; ∗∗, $P < 0.01$; ∗∗∗, $P < 0.001$; NS, not significant, according to t-test from two independent samples.

Figure 2 Shoot dry matter accumulation and stress intensity index (means ± SE) for *P. cathayana* females and males under four growth conditions. PS: P shortage; WS: water shortage; PS+WS: both P and water shortage. Other treatment codes and statistical analyses as in Figure 1.

Figure 3 Rhizosphere citrate concentrations (means ± SE) in *P. cathayana* females and males under four growth conditions. Treatment codes and statistical analyses as in Figure 1.

Figure 4 Composition of main soil microbial groups (means ± SE) in the rhizosphere under four growth conditions (a–f). Data are shown as means ± standard error. Treatment codes and statistical analyses as in Figure 1.

Figure 5 Principal coordination analysis (PCoA) of soil microbial communities in the rhizosphere of *P. cathayana* females (a) and males (b) under four growth conditions. Values on PCoA axes indicate the percentages of total variation explained by each axis

Figure 6 Pearson correlations among main soil microbial groups and rhizosphere citrate concentrations of *P. cathayana* females and males under four growth conditions (a-d).

Figure 1

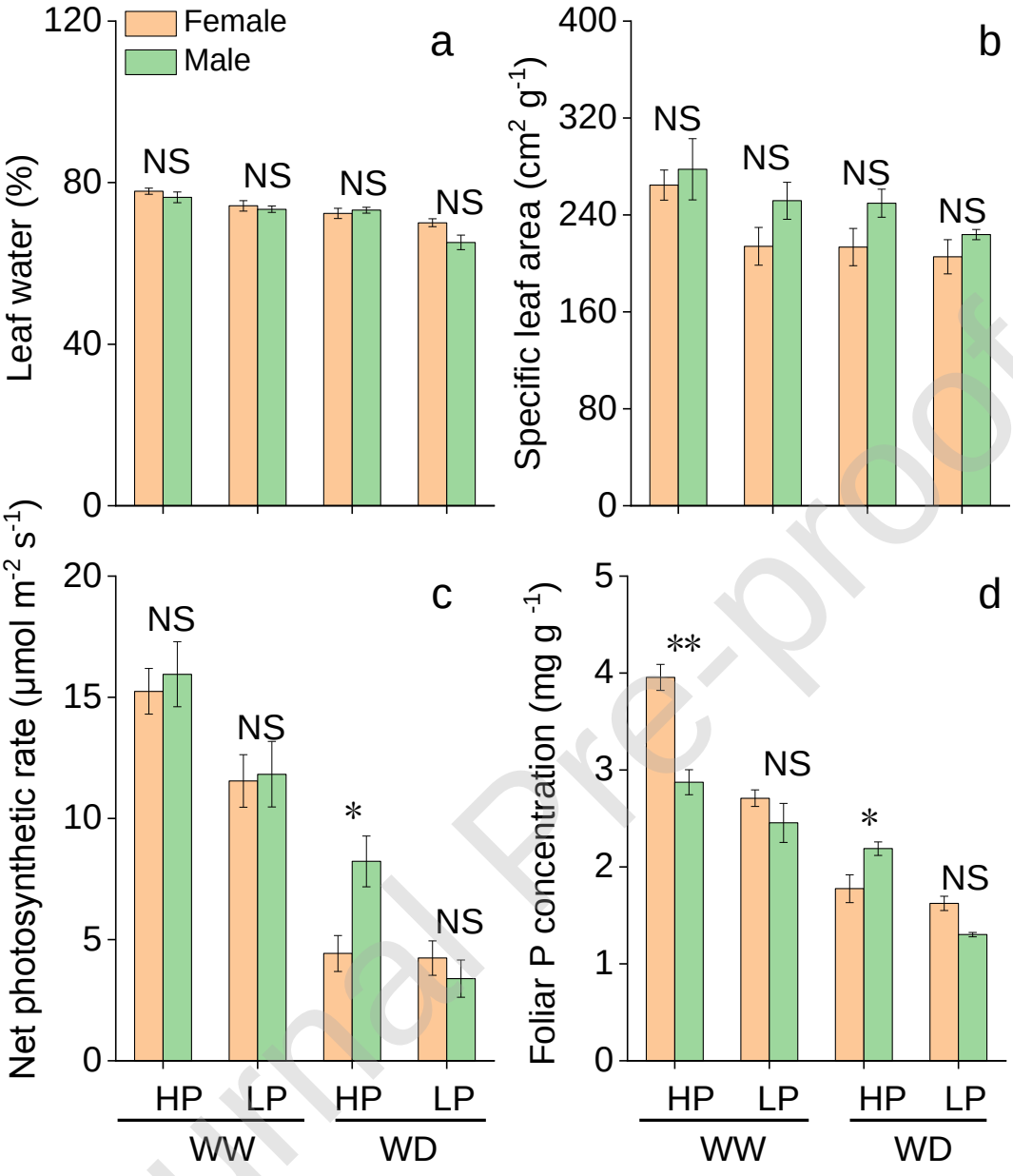
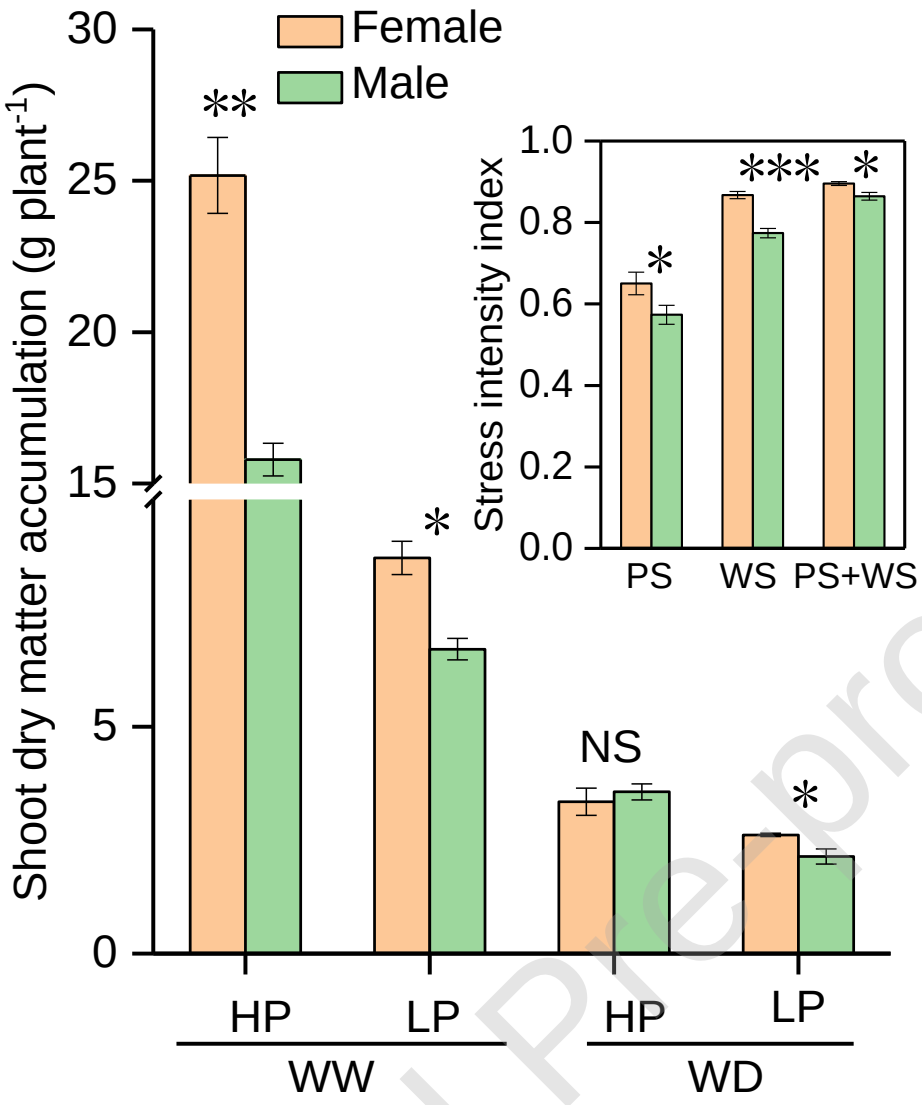


Figure 2



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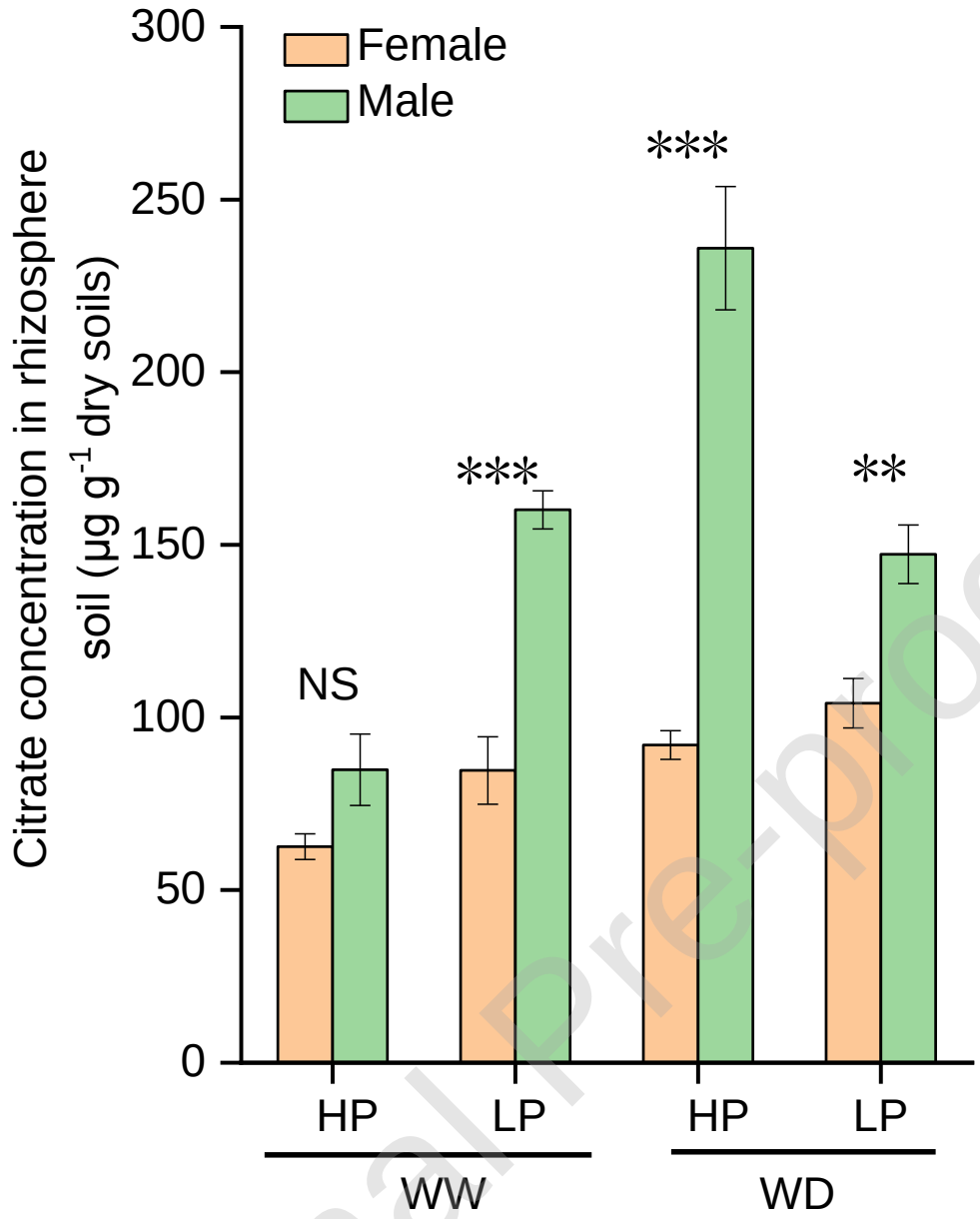

Figure 4

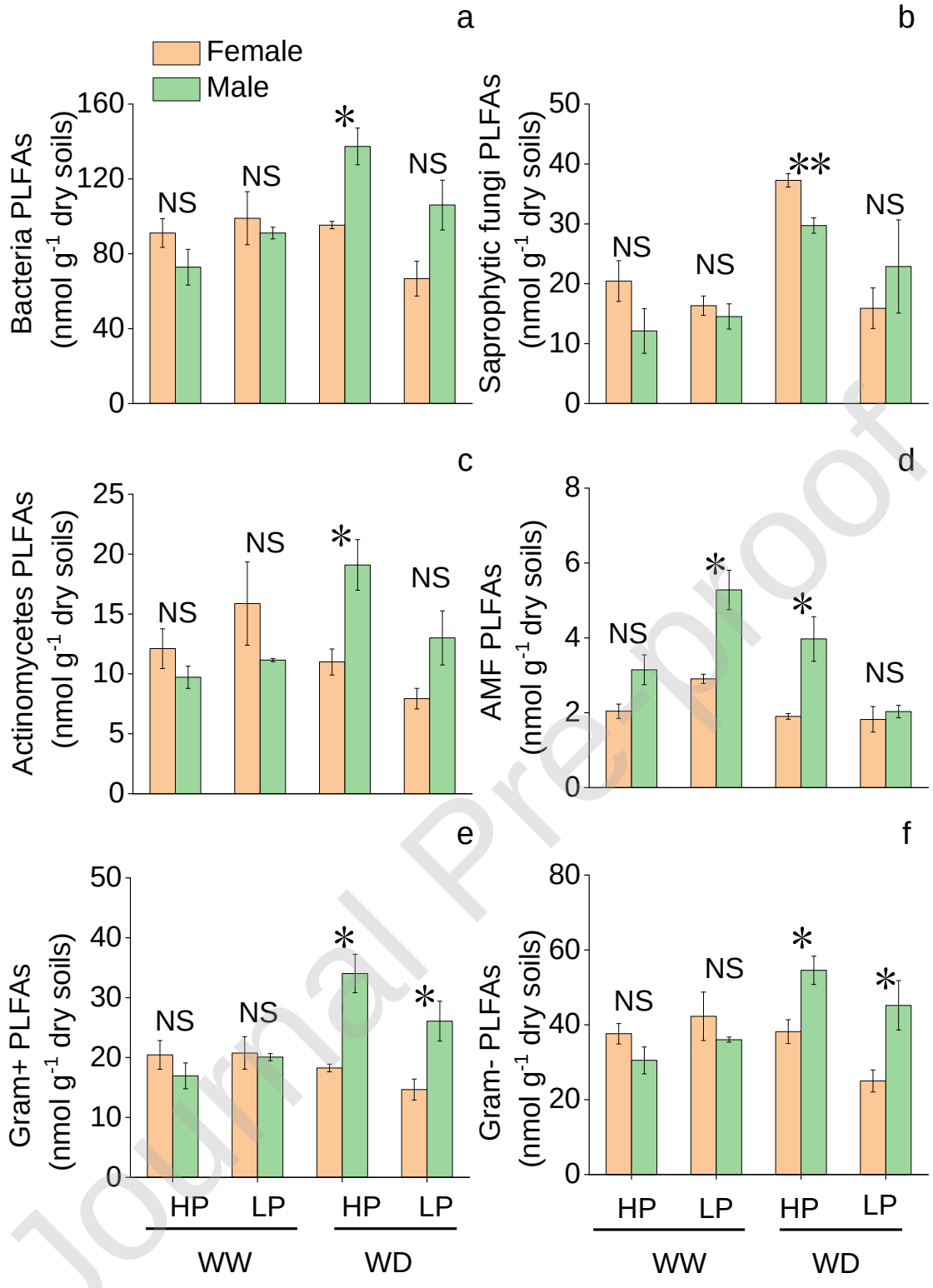

Figure 5

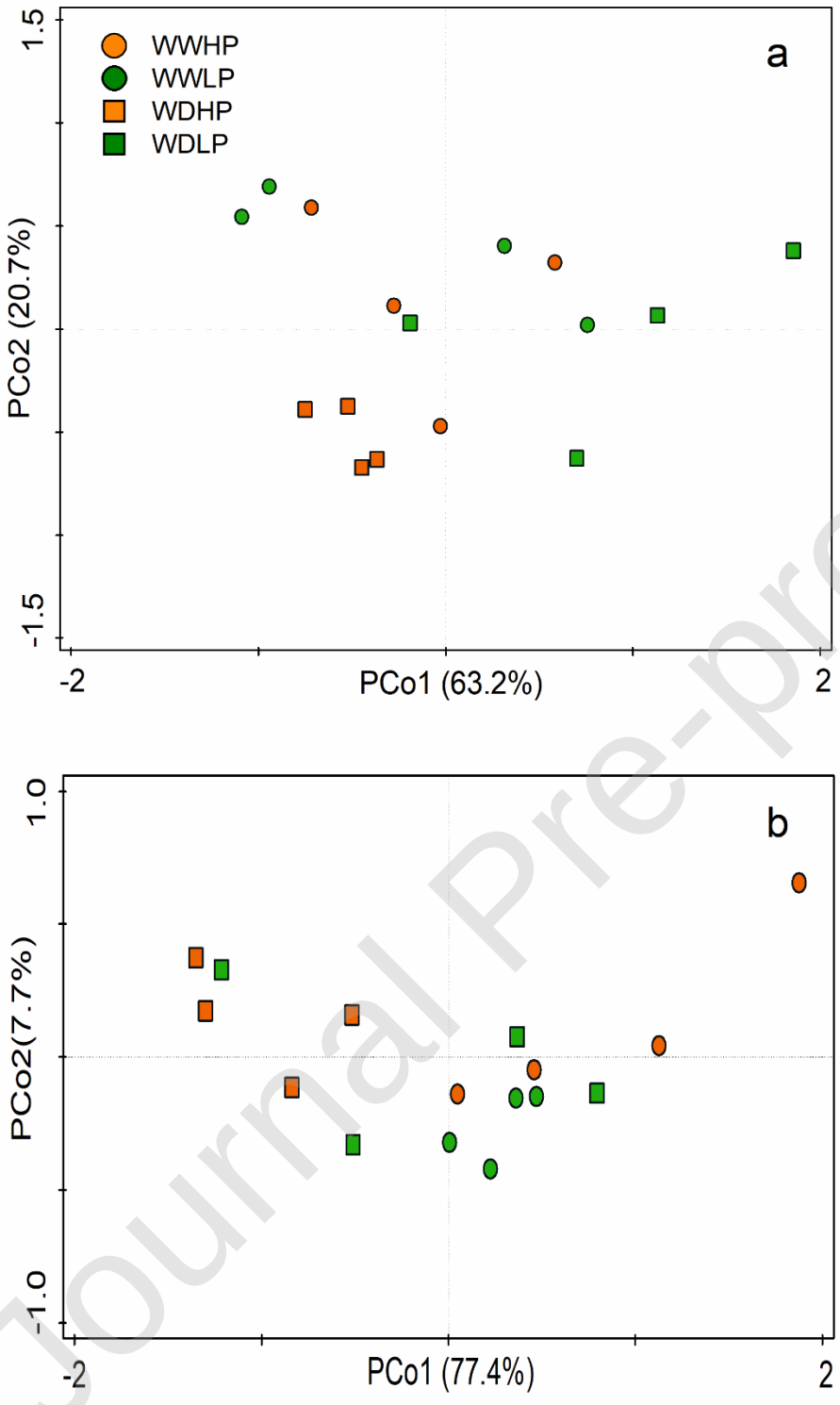

Figure 6